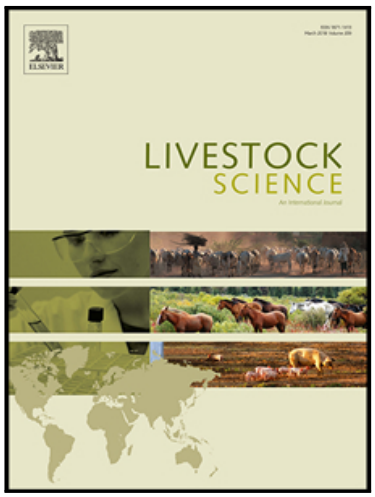

Effect of dietary inclusion of benzoic acid (VevoVitall®) on the microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs

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Highlights

- Benzoic acid limited lactic acid bacteria growth in liquid feed for finisher pigs
- The pH of liquid feed was stabilised with benzoic acid inclusion
- Benzoic acid limited spontaneous fermentation in liquid feed for finisher pigs
- Benzoic acid supplementation did not impact grow-finisher pig growth
- Supplementation of benzoic acid to grow-finisher pigs did not impact carcass traits

Effect of dietary inclusion of benzoic acid (VevoVitall®) on the microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs

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Declarations of interest: none

Abstract

Benzoic acid has long been used as a food preservative due to its antibacterial and antifungal effects. Supplementation to pig diets has also been shown to inhibit microbial free amino acid degradation and to control yeast growth in fermented liquid feed. However, the effect of dietary inclusion of benzoic acid (BA) in fresh liquid feed for grow-finisher pigs on feed quality and the resultant effects on pig growth remain unclear. The objective of the current study was to compare four inclusion levels of BA (VevoVitall®) on feed microbial quality and on the growth performance of grow-finisher pigs. Two-hundred and sixteen pigs with a starting weight of 30.0kg (± 7.43 SD) were used in the experiment. The four dietary treatments were as follows: (1) Basal diet + 0kg/t BA (0kg/t BA), (2) Basal diet + 2.5kg/t BA (2.5kg/t BA), (3) Basal diet + 5kg/t BA (5kg/t BA), (4) Basal diet + 10kg/t BA (10kg/t BA). Lactic acid bacteria (LAB) counts in the mixing tank were similar across treatments ($P>0.05$) but were lower in the troughs for the feed supplemented with 10kg/t BA than for all other treatments ($P<0.01$). The pH of the 10kg/t BA treatment was also lower than that of the other three treatments. However, this only occurred in the mixing tank ($P<0.01$), as in the trough, the basal diet had the lowest pH (lower than the other three treatments; $P<0.01$). Dietary BA inclusion did not affect average daily gain, average daily feed intake, feed conversion efficiency, final live-weight, carcass weight or carcass quality during the experimental period ($P>0.05$). In conclusion, while BA may limit the growth of LAB in liquid feed and stabilise feed pH, its inclusion in the diet did not improve the growth performance or carcass quality of grow-finisher pigs.

Key words: Dietary acidification; fattener; swine; wet feed

Introduction

Benzoic acid (BA) has been authorised as a feed additive for grow-finisher pigs at inclusion levels of 0.5% - 1% in the diet and is included in the functional group of 'other zootechnical additives' (EU regulation No. 1138/2007/EC; EFSA, 2007). The metabolic end product of BA is hippuric acid which can decrease urinary pH, so one of the main reasons for using BA is to reduce ammonia emissions from manure. Benzoic acid is a monocarboxylic acid which is used as an antibacterial and antifungal chemical preservative in the food industry (E-number: E210) (Mao et al., 2019). It has also been shown to reduce the loss of free amino acids (AA) in fermented liquid feed, which occurs via microbial degradation (Vils et al., 2018). This is presumably by inhibition of microbial growth, as the same study also showed an inhibition of yeast growth and a reduction in the amount of lactic acid produced in the benzoic-acid supplemented feed.

Improved feed conversion efficiency (FCE) has also been reported with dietary BA supplementation in grow-finisher pigs (Den Brok, 1999; Van der Peet-Schwering et al., 1999; Øverland et al., 2008) and improved growth rates have been found in weaner pigs (Partanen and Mroz, 1999; Kluge et al., 2006; Guggenbuhl et al., 2007; Torrallardona et al., 2007; Halas et al., 2010; Diao et al., 2016). This enhanced growth performance is most likely due to the antibacterial activity of BA in the pig gut, particularly against coliforms (Knarreborg et al., 2002; Kluge et al., 2006; Øverland et al., 2008; Papatsiros et al., 2011). However, previous work has shown no significant effect of benzoic acid supplementation on carcass quality parameters when supplemented to diets alone (Lenis et al., 1998) oras part of an acid blend (Den Brok, 1999).

The objective of the current study was to compare the effect of four dietary inclusion levels of BA (0kg/t, 2.5kg/t, 5kg/t and 10kg/t) on the microbial quality of liquid feed and on the growth of grow-finisher pigs. It was hypothesised that BA would have an antimicrobial

effect in liquid feed, thereby limiting spontaneous fermentation and improving feed microbial quality. Furthermore, it was hypothesised that increasing dietary BA inclusion would improve growth and feed efficiency in liquid fed grow-finisher pigs.

Materials and methods

Ethical approval

Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC 107/2015). The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

Experimental design and animal management

The experiment used 216 Danavil Duroc x (Landrace x Large White) female and entire male pigs with an initial live-weight (LW) of 30.0kg ± 7.43 SD. Pigs were penned in groups of 6 pigs with a total of 9 pen replicates/treatment. Pen groups were given a 7-day adaptation period to liquid feeding prior to the start of the experiment, during which all were fed a control diet (0 kg/t BA). Pen groups were blocked by sex and weight, with both sexes represented in each block, following which pens were randomly assigned to one of four dietary treatments, as follows; (1) Basal diet, 0kg/tonne VevoVitall® (0kg/t BA); (2) Basal diet + 2.5kg/tonne VevoVitall® (2.5kg/t BA); (3) Basal diet + 5kg/tonne VevoVitall® (5kg/t BA) ; and (4) Basal diet + 10kg/tonne VevoVitall® (10kg/t BA).

All pigs were assigned to dietary treatments on the same day of the experiment (day 0). The heaviest two blocks of pigs were on trial for 56 days and slaughtered on day 57 (108.1kg ± 5.39 SD), while the lighter pigs were on trial for 76 days and slaughtered on days 77 and 78 (118.1kg ± 8.95 SD). Pigs were slaughtered at a mean LW of 115.kg ± 9.2 SD.

Pen groups were housed in pens (2.37m × 2.36m) with concrete slatted floors and solid PVC partitions. Each pen group had access to a water bowl (DRIK-O-MAT, Egebjerg International A/.S, Egebjerg, Denmark) as per regulation Council Directive 2008/120/EC (2008). Air temperature was maintained at 20 to 22°C and was recorded daily. The room was mechanically ventilated with exhaust fans and air inlets controlled by a Steinen PCS

8100 controller (Steinen BV, Nederwert, The Netherlands). Pigs were observed closely twice daily and any pig showing signs of ill-health were treated appropriately. All veterinary treatments were recorded, including identity of pig, symptom, medication and dosage administered.

Each pen was equipped with one solenoid valve above a short liquid feeding trough fitted with an electronic sensor. The electronic sensors were checked, 4 times per day, increasing to 6times per day, after 4 weeks, and additional feed was dispensed into troughs where the residual feed in the trough was below the level of the sensor. Feeding was according to a feeding curve to provide *ad-libitum* access to feed. Feed level in the trough was manually inspected daily before and after feeding and feed allocation per pen increased or decreased accordingly. The short stainless-steel troughs (100 cm × 32.5 cm × 21cm) were located on top of a rubber mat (1.5 × 1 m) which helped to minimise liquid feed wastage.

Diet preparation and feeding

A common diet based on wheat, barley and soybean meal formulated to 9.8 MJ net energy/kg and 9.97g/kg standardised ileal digestible lysine was used. All other AA were supplied relative to lysine according to the ideal protein concept (NRC, 2012). A commercially available BA product (VevoVitall®, DSM Nutritional Products, Basel, Switzerland) was included in the diet at 0 kg/t, 2.5kg/t, 5kg/t and 10kg/t, for treatments 1, 2, 3 and 4 respectively, and directly replaced wheat in the diet. The diets were manufactured in meal form at the Teagasc feed mill (Moorepark, Fermoy, Co. Cork). Ingredient and chemical composition of the diet is shown in Table 1.

The dietary treatments were prepared and liquid-fed using an automatic sensor feeding system (HydroMix, BigDutchman, Vechta, Germany). Diets were prepared in a mixing tank with a 6 pale agitator and agitated for ~5 minutes prior to feed-out. The high-pressure air system delivered liquid feed from the mixing tanks to troughs, each of which was fitted with

a solenoid valve and an electronic feed sensor. If feed was above the sensor in a trough, feed was not dispensed to that particular trough. If feed was below the level of the sensor, feed was dispensed to the trough and sensors were checked automatically before each scheduled feeding. A 12.5 litre rinse of the mixing tanks was carried out after feeding each treatment to prevent contamination from one mix to the next. The water-to-feed ratio used to prepare the liquid feed was 2:1 on a fresh matter basis (FM) or 2.4:1 on a dry matter (DM) basis.

Titrations

Titrations were carried out in order to determine the quantity of BA required to reduce the pH of the diet to 4 as described by Lawlor et al. (2005). Four samples of the basal diet were titrated in duplicate prior to the start of the experiment to determine the amount of acid required to bring the diet to pH 4. Briefly, a 0.5g sample of the diet was added to 50ml deionised water and continuously stirred using a magnetic stirrer. Hydrochloric acid (HCl, 0.1N) was added in 0.2ml increments every 3 minutes and the pH recorded (Mettler Toledo pH meter, Greisensee, Switzerland) prior to the addition of each increment. Four replicates of the VevoVitall® product were also titrated against sodium hydroxide (NaOH, 0.1N) in 1ml increments every 3 minutes to assess how much base would be required to raise the pH to 4. A Pearson square calculation was used to determine the proportions of feed and acid that would produce a diet of pH 4.

Records and sampling

All pigs were weighed on Day 0 and prior to slaughter at the end of the experiment (i.e. day 56 or day 76). Feed disappearance for each pen was recorded daily and average daily gain (ADG), average daily feed intake (ADFI) and FCE were calculated for the entire experiment.

The pH and temperature of liquid feed from each treatment from the mixing tank was recorded using a pH meter (Mettler Toledo) 3 times/week throughout the experiment. To do so, three ~100ml aliquots were removed from the mixing tank during agitation prior to feed-

out and the pH and temperature recorded immediately. The pH and temperature of liquid feed from all 36 troughs was recorded once/week during the experiment, provided feed was available.

Liquid feed samples (~50g) were collected on days 1, 42 and 70 into sterile containers from the mixing tank and 2 troughs/treatment and transported to the laboratory on ice for same-day microbiological analysis. Liquid feed samples for ethanol analysis were collected on day 42 and day 70 from the mixing tank and from 2 troughs/treatment and stored in ~20g aliquots at -20°C until analysis. Dry samples of each diet from each batch of feed produced in the feed mill were pooled into one diet sample per treatment for chemical analysis. Liquid feed samples (~250g) were also collected from the mixing tank (1/treatment) and troughs (2/treatment) on day 42 and day 70 and stored at -20°C for proximate analysis and AA determination.

During exsanguination at the slaughter house, blood samples were collected from 36 pigs (9 pigs/treatment) using Vacuette tubes (Labstock, Dublin, Ireland) for haematological analysis.

Slaughter

Pigs were fasted for ~12 hours prior to slaughter by CO_2 stunning followed by exsanguination in a commercial slaughterhouse. The following measurements were taken: hot carcass weight was recorded 45 minutes after stunning, and back-fat thickness and muscle depth measured at 6cm from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was estimated according to the following formula: Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm) (Department of Agriculture Food and Rural Development, 2001). Cold carcass weight was calculated as hot carcass weight (45 minutes after stunning) × 0.98. Kill-out percentage was calculated from final LW prior to slaughter and cold carcass weight. To calculate

carcass ADG, a kill-out percentage of 65% was applied to LW at the beginning of the experiment and the following equation used: ((carcass weight in kg – LW on day 0 × 0.65) × 1000) / number of days on treatment (Lawlor and Lynch, 2005).

Microbiological analysis of liquid feed

Approximately 10g of each liquid feed sample was homogenized in a stomacher as a 10-fold dilution in maximum recovery diluent (MRD; Oxoid, Basingstoke, UK) and a 10-fold dilution series was performed in MRD. Relevant dilutions were plated in duplicate as follows; (1) pour-plated on de Man Rogosa & Sharpe, (MRS; Merck, Damstadt, Germany) agar, containing 50 U/mL nystatin (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland), overlaid and incubated at 30°C for 72 hours for enumeration of lactic acid bacteria (LAB); (2) pour-plated on violet red bile glucose (VRBG; Oxoid) agar, overlaid and incubated at 37°C for 24 hours for *Enterobacteriaceae;* (3) pour-plated on ChromoCult tryptone bile X-glucuronide (CTBX; Merck) agar and incubated at 44°C for 24 hours for *E. coli*; and (4) spread-plated on yeast glucose chloramphenicol (YGC; Merck) agar and incubated at 25°C for 5 days for yeasts and moulds. Colonies were counted and the counts averaged and presented as $\log_{10}$CFU/g of the original sample.

Feed analysis

The four diets used in the experiment were ground through a 2mm Christy Norris mill and analysed for DM, ash, gross energy (GE) neutral detergent fibre (NDF) ether extract (EE), nitrogen (N) and AA concentration. The DM (AOAC.934.01), ash (AOAC.942.05), and EE concentration (AOAC.920.39) were determined according to methods of the Association of Official Analytical Chemists (AOAC, 2005). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA). The neutral detergent fibre (NDF) content was determined according to the method of Van Soest et al. (1991) using the Ankom 220 Fibre Analyser (Ankom Technology, Macedon, New York, USA). The N

content was determined using the LECO FP 528 instrument (Leco Instruments, UK Ltd., Cheshire, UK) (AOAC.990.0). Crude protein (CP) was determined as N × 6.25. Amino acid determination was carried out using cation exchange HPLC as previously described by McDermott et al. (2016) (AOAC 994.12).

Liquid feed samples collected from the mixing tank and troughs on day 42 and day 70 were oven-dried at 55°C for 72 hours and milled through a 2mm screen using a Christy Norris mill. These samples were pooled prior to analysis to give one mixing tank and one trough sample per treatment which were analysed for GE, N, CP, ash and AA as above.

Preparation of liquid feed samples for ethanol analysis was carried out as described by van Winsen et al. (2000). Briefly, feed aliquots were defrosted prior to centrifugation at 2000g for 10 minutes at 4°C. The supernatant was then centrifuged at 18 500g for 10 minutes. The resulting supernatant was filtered through a 0.2 µm filter and stored at -20°C until analysis. Samples were thawed slowly at room temperature prior to ethanol analysis by gas chromatography (Agilent 6890; Agilent Technologies, Waghaeusel-Wiesental, Germany) using a flame ionization detector. A 1 µL volume of each sample was injected by split injection 5:1 onto the column (AT-100 15 m × 0.53 mm i.d. × 1.2 micron) with a column flow rate of 3.4ml/min helium. The temperature programme was 40°C for 3 minutes, ramped at 10°C/min to 180°C and held at 180°C for 3 minutes.

Haematological analysis of blood samples

Blood samples for haematology were analysed on the day of slaughter using an Abbot Cell-Dyn 3700 analyser (GMI-Inc., Minnesota, USA). The following parameters were measured; white blood cells, neutrophil number and percentage, lymphocyte number and percentage, eosinophil number and percentage, monocyte number and percentage, basophil number and percentage, red blood cells, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and platelets.

Statistical analysis

Growth parameters [ADFI, ADG, FCE and LW], carcass quality parameters and haematology data were analysed using the MIXED procedure of SAS®9.4 (Sas Institute, Inc., Cary, NC, US) with pen as the experimental unit. For growth parameters; treatment, sex and their associated interactions were included in the model as fixed effects. Initial LW and number of days on trial were included in the model as co-variates and pen nested within block was included as a random effect. For carcass quality parameters, carcass growth parameters and haematological analysis; treatment, sex and their associated interactions were included in the model. Carcass cold weight was used as a co-variate for analysis of muscle depth, fat depth and lean meat percentage and initial LW was used as a co-variate for cold weight. Orthogonal linear and quadratic contrast statements were constructed to compare treatment means for growth parameters and carcass quality. Microbial counts were log transformed prior to analysis. The microbial counts, pH and temperature on days 1, 42 and 70 were also analysed using the MIXED procedure of SAS with treatment, sampling location and their associated interaction included in the model as fixed effects and day of sampling included as a random effect. The normality of scaled residuals was investigated using the UNIVARIATE procedure of SAS. Results are presented as least square means ± SEM. Differences were considered significant at $P<0.05$ and as tendencies $0.05<P<0.10$. The MEANS procedure was also used to calculate means and standard deviations for the weekly pH trough recordings carried out.

Results

Pig deaths and removals

Seven pigs were removed from treatment throughout the experimental period and their weights were taken into account in feed intake and growth calculations. This included two pigs from the 0kg/t inclusion rate of BA, one due to suspected stomach ulcers and one due to a prolapse; one pig from the 2.5kg/t BA treatment due to a rupture; three pigs from the 5kg/t BA treatment, one due to a suspected heart attack, one due to pneumonia and one due to a burst rupture; one pig from the 10kg/t BA treatment due to a burst rupture.

Titrations of benzoic acid product against HCl and NaOH (data not shown)

Firstly, it was found that, on average, 0.55ml 0.1N HCl was required to bring the pH of the basal diet to pH 4 which equated to 110mEq/g feed. Titrations of the VevoVitall® benzoic acid additive against the NaOH determined that 8ml NaOH was required to raise the pH of the VevoVitall® product to pH 4 which equated to 1600mEq/g. The Pearson square calculation showed that a ratio of 93.57% feed to 6.43% VevoVitall® was optimal to achieve a pH of 4 with this diet.

Effect of benzoic acid on the microbiological quality, pH and temperature of liquid feed for grow-finisher pigs

The effect of sampling location by treatment interactions on the microbial counts, pH and temperature of liquid feed are shown in Table 2. A sampling location × treatment interaction was observed for LAB. Counts of LAB were lower in troughs of the 10kg/t BA treatment than in troughs of all other treatments, but LAB counts in the mixing tank were not influenced by BA inclusion rate. A tendency for a sampling location × treatment interaction was observed for *Enterobacteriaceae* counts. In liquid feed from the 2.5kg/t BA treatment, counts of *Enterobacteriaceae* were higher in the trough than in the mixing tank; however, counts in the mixing tank and trough were similar at all other BA inclusion rates. There was

also a sampling location × treatment interaction for pH. In the mixing tank, the pH of the 10kg/t BA treatment was lower than the 0kg/t BA and 2.5kg/t BA treatments, whereas in the trough, the pH of 0kg/t BA was lower than for all other treatments. There were no sampling location × treatment interactions for *E. coli*, yeast, or mould counts or feed temperature.

The main effect of treatment must also be considered. In the mixing tank, counts of LAB, *E. coli*, yeast and mould and liquid feed temperature were similar for all treatments. Counts of *Enterobacteriaceae* in the 2.5kg/t BA treatment tended to be lower than those in the 0kg/t BA and 5kg/t BA treatments (P=0.06) but the same as the 10kg/t BA treatment (P>0.05). The pH of the 10kg/t BA treatment in the mixing tank was lower than all other treatments (P<0.05).

There were no differences in *E. coli*, yeast or mould counts in feed samples collected from the troughs; however, counts of LAB were lower in the 10kg/t BA treatment than in the other three treatments (P<0.01). *Enterobacteriaceae* counts tended to be lower in the 10kg/t BA treatment than the 2.5kg/t BA treatment (P=0.06), but were similar to those in the 0kg/t BA and 5kg/t BA treatments (P>0.05). In the pen troughs the opposite occurred in terms of pH, with the pH of the 0kg/t BA treatment lower than that of the other three treatments (P<0.01). A tendency for a treatment effect on temperature was observed in troughs (P=0.07); however, there were no pairwise differences between treatments (P>0.10).

Figure 1 shows the pH of liquid feed from the mixing tank (28 recordings in total; 3 recordings weekly) and troughs (9 recordings in total; 1 recording weekly from 9 troughs/treatment where feed was available) of each treatment. The mean pH of liquid feed from the 28 recordings from the mixing tank of each treatment throughout the trial period was 6.14, 5.93, 5.72 and 5.41 for treatments 1 through 4, respectively. The mean pH of liquid feed from 9 recordings from the troughs of each treatment was 5.12, 5.26, 5.29 and 5.40 for treatments 1 through 4, respectively. The results of these mixing tank and trough

recordings are similar to those reported in Table 3 on days 1, 42 and 70 when liquid feed was microbiologically analysed. The mean temperature of liquid feed from 28 recordings from the mixing tank of each treatment was 20.6°C, 20.4°C, 20.5°C and 20.7°C for treatments 1 through 4, respectively. The mean temperature of liquid feed from 9 recordings of liquid feed from troughs was 22.1°C, 22.0°C, 22.3°C and 22.3°C for treatments 1 through 4, respectively.

Proximate and amino acid analysis of feed

The results of chemical analyses of the dry diets and liquid feed from the mixing tank and troughs of each treatment are shown in Table 3. There were no obvious differences in GE content of the diet between dry and liquid (mixing tank/ trough) feed. There appears to be a loss of lysine in all four treatments when dry and liquid (mixing tank and trough) feed are compared; however, the loss of methionine from the liquid feed in the mixing tank appear greater in the 0kg/t BA treatment than in the other three treatments. Ethanol was not detected in liquid feed sampled from the mixing tank in any treatment on either day 42 or day 70 of the experiment (data not shown). Ethanol concentrations in liquid feed troughs on day 70 were 20.1mM, 1.8mM and 0.5mM from the 0kg/t, 5kg/t and 10kg/t BA treatments respectively. Insufficient sample was available to determine the ethanol content of the 2.5kg/t BA treatment.

Effect of dietary benzoic acid on the growth, feed efficiency and carcass quality of grow-finisher pigs

The effect of treatment on pig growth, feed efficiency and carcass quality is shown in Table 4. No treatment × sex interactions were observed for any of the growth performance parameters or carcass quality traits measured in the current study. There were also no treatment differences observed for ADFI, ADG, FCE, slaughter weight, carcass ADG or

carcass FCE during the experiment (P>0.05). Despite tendencies towards a difference in carcass ADG (P=0.07) and carcass cold-weight (P=0.09), no pairwise differences were observed between treatments. Similarly, no treatment differences were observed for kill-out percentage, muscle depth, fat depth or lean meat percentage at slaughter (P>0.05). There were no significant linear or quadratic effects for growth performance parameters or carcass quality traits in the current study.

Effect of dietary benzoic acid on the haematological profile of pigs at slaughter

Results from the haematological analysis are shown in Table S1. There were no significant treatment × sex interactions for any of the haematological parameters measured (P>0.05). The only treatment effect observed was that pigs fed the 0kg/t BA treatment tended to have a lower number of platelets than pigs fed the 2.5kg/t BA treatment (P=0.09). Platelet counts were slightly below the normal range of 200 – 500 cells x10^9/L reported in the Merck manual in pigs fed the 0kg/t treatment.

Discussion

Benzoic acid has long been used as a food preservative due to its antibacterial and antifungal activity (Mao et al., 2019). It can also be used as a feed additive; however, most of the research to date on dietary inclusion of BA has been performed in dry or wet/dry feed (Den Brok, 1999; Guggenbuhl et al., 2007; Torrallardona et al., 2007). To our knowledge, this is the first investigation of the impact of BA both on the microbial quality of fresh liquid feed and growth of grow-finisher pigs in the same study. Our objective was to improve the microbial quality of liquid feed by adding BA to the diet and, consequently to improve pig growth.

While dietary BA inclusion did not affect overall or carcass growth or feed efficiency in the current study, it is important to note that the growth rate was exceptionally high and feed efficiency was extremely good for pigs on all treatments. For this reason, it would have been difficult to obtain a biological improvement in ADG, FCE or both in response to dietary inclusion of BA. Management of the liquid feeding system was extremely good in the current experiment, in an attempt to minimise wastage which was previously found to be the most likely cause of poorer FCE when liquid feeding (Russell et al., 1996; l'Anson et al., 2012). It is evident from the growth rates and FCEs achieved that feed wastage was minimised while still ensuring *ad-libitum* access to feed by the pigs in the current study.

The benefits of dietary BA inclusion are not as pronounced in older pigs as in younger pigs (Bühler, 2009). It has been suggested that as pigs age, the BA supplementation-mediated improvement in digestive ability from the associated pH reduction and the increase in activity of digestive enzymes declines (Diao et al., 2016). This, combined with the excellent feed efficiency found for the control group in the current experiment help to explain why no treatment differences in pig growth and feed efficiency were found in the current experiment. While no studies, to date have investigated the growth performance of pigs fed fresh liquid

feed supplemented with BA, Van der Peet-Schwering et al. (1999) reported a 0.1 unit improvement in FCE when dry-feeding 10 kg/t BA to finisher pigs. However, the FCE for the control diet in the Van der Peet-Schwering et al. (1999) was 2.78, whereas it was 2.27 in the present study. Hence, it would appear that, there was greater scope for FCE to improve due to dietary BA inclusion than in the current study.

The results of this study show that, although dietary BA inclusion did not influence pig growth, at 10kg/t BA inclusion it did stabilise liquid feed pH from the mixing tank to the troughs and reduced the growth of LAB in residual feed in the troughs. This resulted in the highest pH recording in the troughs of the 10kg/t BA treatment, despite the fact that the opposite was true in the mixing tank, where the lowest pH was recorded in the treatment with the highest inclusion level of BA. It is well known that the production of lactic and acetic acids by LAB and yeasts in liquid feed reduces the pH of the mixture (Missotten et al., 2015). The reduced growth of LAB in the 10kg/t BA treatment while in the feed trough therefore seems to have reduced microbial acid production. The pKa value of BA is 4.19, while that of lactic acid is 3.86 (Schutte, 2011). A lower pKa value generally equates to a stronger acid, so it is not surprising that when the LAB count and, as a result, lactic acid concentration increased in the troughs of the 0kg /t BA treatment that a greater pH reduction was observed in the troughs of this treatment compared with the other three treatments. The potential of BA to inhibit yeast growth and lactic acid production in liquid (fermented) feed has previously been reported (Vils et al., 2018).

The pH reduction and increase in LAB counts and ethanol concentrations between the mixing tank and troughs for the control diet without BA suggest that spontaneous fermentation was occurring (Scholten et al., 1999) in the present study while feed resided in the troughs. Our results suggest that 1% dietary BA inclusion prevented this spontaneous fermentation, as

evidenced by the stabilised pH and limited growth of LAB over time compared to the control diet (although there was no effect on yeast growth).

Despite the fact that enteric bacteria are reportedly reduced in the gut by BA supplementation (Kluge et al., 2006; Guggenbuhl et al., 2007), no BA-mediated reduction in *Enterobacteriaceae* was found in the liquid feed in the present study. This is most likely because the pH reduction achieved in the liquid feed was not sufficient. A pH of ~4.0 is required to reduce coliform counts in liquid feed (Geary et al., 1999; Plumed-Ferrer et al., 2004) and pH 4.72 was the lowest pH recorded in our study.

Amino acid analysis from the current study shows a loss of lysine in the mixing tanks and troughs of all four treatments when compared with the dry feed; however, the biggest loss appears to be in the control treatment without BA supplementation. This suggests that the BA might be preventing microbial degradation of free AA as a result of controlling spontaneous fermentation, in agreement with previous findings for fermented liquid feed (Vils et al., 2018). However, the results from the current study should be treated with caution, as the liquid feed data are from only one pooled sample at each location.

No treatment differences were observed in the haematological profile of pigs in the current study, except that the platelet count tended to be higher in pigs supplemented with 2.5g/t BA. This is likely due to the fact that the count was slightly below the normal range in the non-BA supplemented pigs. Overall, the findings show that, although white blood cell counts were slightly higher than the normal range in the 2.5kg/t BA and 5kg/t BA treatments, and the mean corpuscular haemoglobin was slightly above the normal range in all BA-supplemented pigs (Jackson and Cockcroft (2008), health status was not impacted by BA inclusion in the diet.

The upper dietary benzoic acid inclusion limit for pigs is 10kg/t (EU regulation No. 1138/2007/EC). The titrations conducted as part of the current study suggested that if we wish to reduce the pH of liquid feed to 4, to help reduce *Enterobacteriaceae* counts, that 64.3kg BA/tonne feed would be required. Previous work has shown that increasing dietary inclusion from 10kg/t to 20kg/t worsened pig growth rate and feed efficiency (Van der Peet-Schwering et al., 1999) and hence controlling feed hygiene using BA is unlikely to be feasible.

Conclusion

Findings from the present study showed that dietary inclusion of BA at 10kg/t reduced LAB growth and the associated reduction in feed pH while feed resided in the feed trough, indicating that spontaneous microbial fermentation was somewhat controlled. However, dietary BA supplementation did not improve growth or feed efficiency in liquid-fed grow-finisher pigs, most likely because the growth and feed efficiency of unsupplemented pigs was already very high.

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Figure caption

Figure 1. pH of liquid feed for grow-finisher pigs containing four dietary inclusion rates of benzoic acid (VevoVitall ®; 0kg/t, 2.5kg/t, 5kg/t and 10kg/t) sampled from both the mixing ta

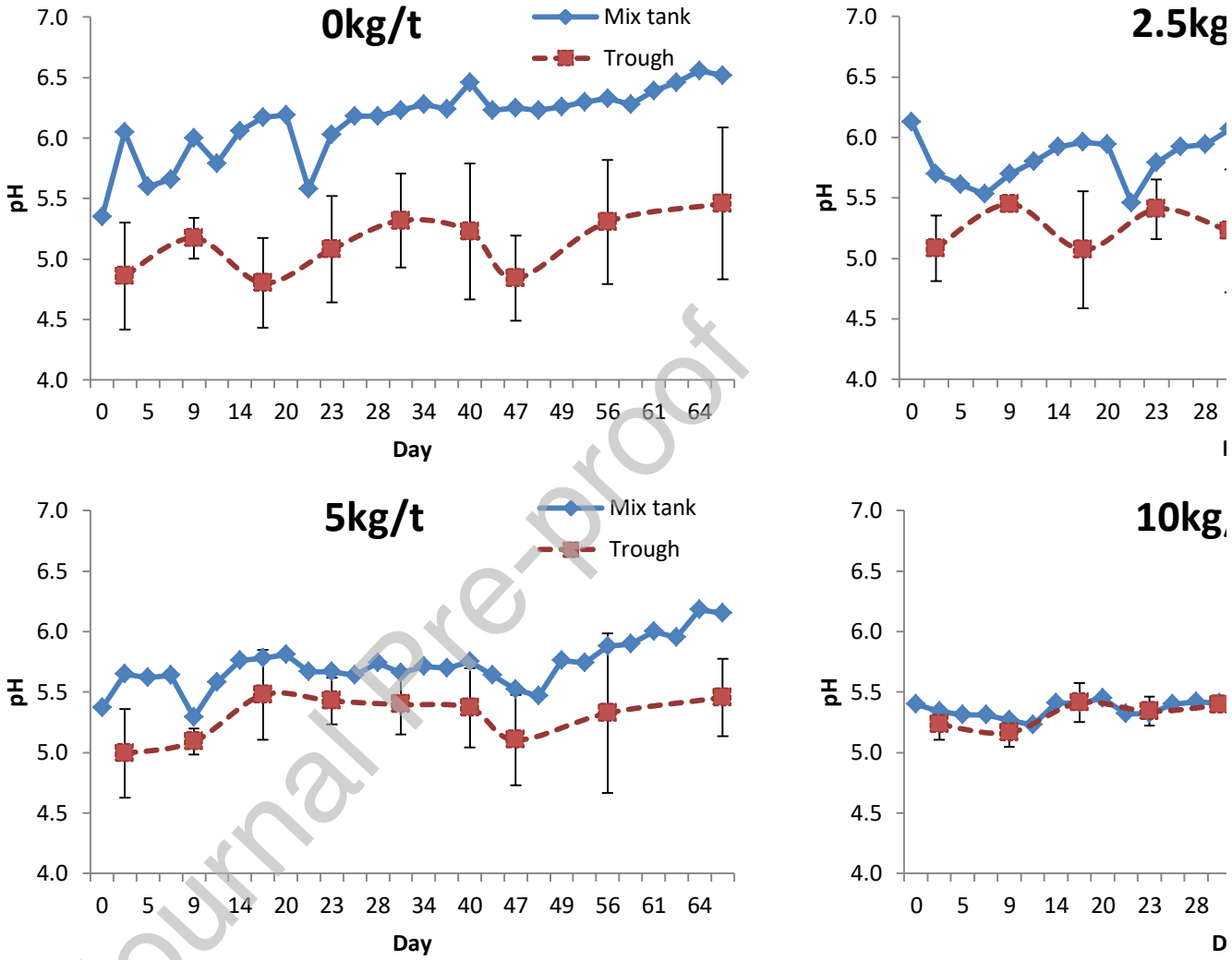

Figure 1. pH of liquid feed for grow-finisher pigs containing four dietary inclusion rates of benzoic acid (VevoVitall ®; 0kg/t, 2.5kg/t, 5kg/t and 10kg/t) sampled from both the mixing tank and troughs

Tables

Table 1. Ingredient and chemical composition of the experimental diets (on an as-fed basis, g/kg unless otherwise stated)

	Inclusion rate of benzoic acid (kg/t)			
	0.0	**2.5**	**5.0**	**10.0**
Ingredient composition				
Wheat	400.0	397.5	395.0	390.0
Barley	382.7	382.7	382.7	382.7
Soya bean meal	183.0	183.0	183.0	183.0
Benzoic acid[1]	0.0	2.5	5.0	10.0
Limestone flour	11.0	11.0	11.0	11.0
Lysine HCl	3.8	3.8	3.8	3.8
Mono DiCalcium phosphate	1.0	1.0	1.0	1.0
Salt	3.0	3.0	3.0	3.0
L-Threonine	1.7	1.7	1.7	1.7
Soya oil	9.7	9.7	9.7	9.7
Vitamin and mineral pre-mix[2]	1.0	1.0	1.0	1.0
DL-Methionine	0.9	0.9	0.9	0.9
Celite	2.0	2.0	2.0	2.0
L-Tryptophan	0.2	0.2	0.2	0.2
Phytase[3]	0.1	0.1	0.1	0.1
Chemical composition				
Dry matter	880.0	880.0	881.0	880.0
Crude protein	182.0	175.0	179.0	175.0

	Inclusion rate of benzoic acid (kg/t)			
	0.0	**2.5**	**5.0**	**10.0**
Ash	37.7	35.4	40.4	42.3
Oil	40.6	35.3	36.1	41.7
Neutral detergent fibre[4]	138.0	138.0	138.0	138.0
Gross energy, MJ/kg	15.7	16.2	16.2	16.2
Lysine	10.8	10.8	10.8	10.8
Methionine	4.4	4.3	4.3	4.3
Threonine	7.2	8.1	7.3	7.0
Digestible energy, MJ/kg[4]	13.8	13.8	13.8	13.8
Net energy, MJ/kg[4]	9.8	9.8	9.8	9.8
SID[5] lysine[4]	9.97	9.97	9.97	9.97
Total calcium[4]	6.59	6.59	6.59	6.59
Digestible phosphorus[4]	2.6	2.6	2.6	2.6

[1] VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)

[2] Vitamin and mineral premix provided per kilogram of complete diet: Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; retinyl acetate, 0.7mg; cholecalciferol, 12.7 µg; DL-alpha-tocopheryl acetate, 40 mg; Vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10mg; vitamin B1, 2 mg; vitamin B6, 3 mg and celite 2000mg/kg.

[3] The diet contained 500 phytase units (FYT) per kg feed from RONOZYME HiPhos (DSM, Belfast, UK)

[4]Calculated values

[5]SID: Standardised ileal digestible

1

Table 2. Effect of sampling location (mixing tank or trough) and dietary benzoic acid inclusion rate on the microbial quality, pH and temperature of liquid feed[1,2]

Location	Mixing tank				Trough					P-value		
Benzoic acid, kg/t[3]	**0.0**	**2.5**	**5.0**	**10.0**	**0.0**	**2.5**	**5.0**	**10.0**	SEM	**Location x Treatment**	**Location**	**Treatment**
Lactic acid bacteria[4]	$6.76^{a,b}$	5.65^{a}	$6.47^{a,b}$	6.11^{a}	9.15^{c}	8.65^{c}	8.72^{c}	7.42^{b}	0.258	0.04	0.001	0.01
Enterobacteriaceae[4]	$5.32^{A,B}$	4.70^{A}	$5.28^{A,B}$	$4.96^{A,B}$	$5.36^{A,B}$	5.52^{B}	$5.24^{A,B}$	$4.84^{A,B}$	0.178	0.07	0.18	0.12
E. coli[4]	2.15	2.00	2.00	2.00	3.61	3.81	3.51	3.06	0.243	0.50	0.001	0.42
Yeast[4]	4.33	3.91	3.89	4.09	6.26	6.38	6.17	5.95	0.157	0.21	0.001	0.31
Mould[4]	3.15	3.09	3.08	3.08	3.66	3.64	3.51	3.27	0.182	0.70	0.01	0.56
pH	6.2^{d}	6.0^{d}	$5.9^{c,d}$	$5.5^{b,c}$	4.7^{a}	$5.4^{b,c}$	5.2^{b}	$5.5^{b,c}$	0.17	0.001	0.001	0.08
Temperature, °C	19.6	19.5	19.8	19.6	19.4	19.8	21.5	21.2	1.45	0.40	0.09	0.27

[1]Least square means and pooled standard errors of the mean

[2] Results are the mean of data obtained on day 1, day 42 and day 70 of the experiment

[3] VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)

[4]Counts in $\log_{10}$ CFU/g

[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P<0.05$)

[A,B,C] Within each row, values that do not share a common superscript tend to be different ($0.05<P<0.10$)

Table 3. Gross energy, crude protein, ash and amino acid analysis of dry diets and liquid feed from the mixing tank and troughs containing different inclusion rates of benzoic acid (presented on a DM basis)[1]

Sampling location		**Mixing tank**				**Trough**			
Benzoic acid (kg/t)[2]	**Dry[3]**	**0.0**	**2.5**	**5.0**	**10.0**	**0.0**	**2.5**	**5.0**	**10.0**
Gross energy, MJ/kg	18.3	18.7	18.8	18.7	18.4	18.4	18.7	18.1	18.4
Crude protein, %	20.2	22.1	22.2	23.2	21.3	19.2	21.9	21.1	21.0
Ash, %	4.42	3.83	4.20	4.05	4.07	5.04	5.43	6.49	5.33
Amino acids, g/kg									
Lysine	12.4	8.6	10.4	10.7	9.8	8.2	8.4	NT[4]	8.4
Methionine	4.9	4.5	5.1	5.4	5.1	4.5	4.7	NT	4.9
Threonine	8.4	7.7	8.9	9.4	9.0	7.7	8.3	NT	8.7
Cysteic acid	5.9	5.3	5.9	6.2	6.1	5.7	5.0	NT	5.3
Taurine	1.4	1.5	1.3	1.5	2.3	1.6	3.4	NT	3.6
Aspartic acid	19.1	16.5	19.5	20.7	20.1	16.5	17.8	NT	19.3

Sampling location		**Mixing tank**				**Trough**			
Benzoic acid (kg/t)[2]	**Dry[3]**	**0.0**	**2.5**	**5.0**	**10.0**	**0.0**	**2.5**	**5.0**	**10.0**
Serine	10.2	9.2	10.5	11.0	10.8	9.4	9.6	NT	10.1
Glutamic acid	46.7	43.2	47.5	49.1	48.5	45.2	44.7	NT	49.0
Glycine	9.0	8.2	9.3	9.7	9.5	8.5	8.7	NT	9.5
Alanine	8.5	7.7	8.9	9.1	8.9	8.2	7.7	NT	8.6
Cysteine	0.7	0.9	1.0	0.9	0.8	0.9	1.4	NT	2.7
Valine	10.2	9.2	10.4	11.0	10.5	9.6	9.2	NT	11.5
Isoleucine	7.9	7.6	8.6	9.1	8.8	7.7	8.0	NT	8.7
Leucine	15.1	13.6	15.3	16.3	15.9	13.9	14.4	NT	15.4
Tyrosine	5.6	5.3	6.1	6.5	6.2	5.2	3.9	NT	4.5
Phenylalanine	10.4	9.4	10.5	11.1	10.8	9.9	9.6	NT	10.2
Histidine	6.2	5.4	6.1	6.6	6.5	5.4	6.2	NT	6.8
Arginine	12.7	11.0	12.6	13.7	13.2	11.0	11.5	NT	12.2
Proline	14.7	14.0	14.5	15.3	15.2	15.3	15.5	NT	14.9

[1]Results are from pooled samples: Dry sample pooled from 3 feed batches from the mill for each diet (n=3/treatment prior to pooling); Mixing tank sample pooled from 1 sample/treatment on day 42 and 1 sample/treatment on day 70 for each treatment (n=2/treatment prior to pooling); Trough sample pooled from 2 samples/treatment on day 42 and 2 samples/treatment on day 70 (n=4/treatment prior to pooling).

[2]Mean results for 4 dry diets

[3]VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)

[4]NT=Not tested

Table 4. Effect of four dietary inclusion rates of benzoic acid on the growth, feed efficiency and carcass quality of liquid-fed grow-finisher pigs[1]

	Inclusion rate of benzoic acid (kg/t)[2]							P-
	0	2.5	5	10	SEM	Treatment	Sex	Treat
No pens/trt[3]	9	9	9	9				
Start-weight, kg	36.0	35.3	36.8	37.1	1.87	0.72	0.03	
ADFI[3], g/day	2846	2930	2829	2900	69.3	0.61	0.64	
ADG[3], g/day	1226	1262	1239	1269	21.7	0.18	<0.001	
FCE[3], g/day	2.27	2.29	2.23	2.24	0.034	0.609	<0.001	
Slaughter weight, kg	117.2	118.9	115.9	120.1	8.38	0.11	<0.001	
Carcass								
ADG[4], g/day	901	928	901	919	12.9	0.07	<0.001	
FCE[5], g/g	3.00	3.02	3.04	3.05	0.05	0.937	<0.01	
Cold-weight, kg	86.1	87.3	84.8	87.6	5.64	0.09	0.01	
Kill-out, %	74.1	74.0	73.7	73.4	0.89	0.75	<0.001	
Muscle, mm	46.9	47.5	48.1	48.3	0.53	0.12	<0.001	
Fat, mm	13.8	14.3	13.7	13.3	0.36	0.30	<0.001	
Lean meat, %	55.5	55.2	55.8	56.1	0.31	0.19	<0.001	

[1] Least square means and pooled standard errors of the mean. There were 9 pen replicates per treatment with 6 pigs per pen replicate

[2] VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)

[3] ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency

[4] Carcass ADG: From weight at start of experiment to slaughter = ((carcass weight in kg – LW on day 0 × 0.65) × 1000) / number of days on treatment (Lawlor and Lynch, 2005)

[5] Carcass FCE: From start of experiment to slaughter = total average daily feed intake / carcass ADG (g)

Credit author statement

F.M. O' Meara: Investigation, formal analysis, writing - original draft

G.E. Gardiner: Supervision, writing - review & editing, methodology, validation

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